Volatile and non-volatile compounds in green tea affected in harvesting time and their correlation to consumer preference

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Abstract Current study was designed to find out how tea harvesting time affects the volatile and non-volatile compounds profiles of green tea. In addition, correlation of instrumental volatile and non-volatile compounds analyses to consumer perception were analyzed. Overall, earlier harvested green tea had stronger antioxidant capacity (~61.0%) due to the polyphenolic compounds from catechin (23,164 mg/L), in comparison to later harvested green teas (11,961 mg/L). However, high catechin content in green tea influenced negatively the consumer likings of green tea, due to high bitterness (27.6%) and astringency (13.4%). Volatile compounds drive consumer liking of green tea products were also identified, that included linalool, 2,3-methyl butanal, 2-heptanone, (E,E)-3,5-Octadien-2-one. Finding from current study are useful for green tea industry as it provide the difference in physiochemical properties of green tea harvested at different intervals.

Keywords Green tea · Catechin · Volatile and non-volatile analysis · Sensory analysis

Introduction

Tea (*Camellia sinensis*) is well-accepted and most consumed drink in the world (Kin et al. 2011; Yanagimoto et al. 2003). Due to its distinct flavor characteristics and health promoting properties, the tea consumption has been steadily increasing throughout the world (Kin et al. 2011; Rietveld and Wiseman 2003). The consumption pattern of tea is slightly different according to the region, in that the consumption of black tea is common in Western countries, whereas the consumption of green tea is more dominant in Asia (Yanagimoto et al. 2003). Recently, the health benefits associated with drinking tea were reported, that included lowering a risk of getting cardiovascular disease (Chan et al. 1999; Cheng 2006), antimutagenic effect (Chen et al. 2001; Kuroda and Hara 1999), anticarcinogenic (Stoner and Mukhtar 1995; Chung et al. 1997) and antioxidative effect (Yanagimoto et al. 2003; Nakagawa et al. 2002; Toschi et al. 2000).

Currently, Korean green tea products are generally priced based on the tea leaf harvesting time. Harvesting time of tea leaf is one of the most important factors influencing the chemical, physiological and physical qualities of tea, along with geographical difference and processing method. Even if the tea leaf was grown in the same area and processed by same method, teas have different names and prices based on its harvesting time, which it has been long-believed that harvesting time is an important factor for determining tea quality. In the case of Korean green tea market, green tea leaves are collected between April and May in Korea. The tea leaves collected before April 20th are considered as the highest quality grades due to its rarity, characteristic taste, and softness (called Woojeon), tea leaves collected between April 20th and April 30th, are called Gokwoo, tea leaves collected in

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early May, are called Sejak, and the tea leaves collected after mid-May are called Choongjak, which is believed to have lowest quality among these four teas (Lee et al. 2014). Price for green tea has been established based on the harvesting time of the year, in which the believed quality is based on. After harvesting, all leaves are proceeded into the fermentation process except white and green teas that are only dried and packaged for the commercialization.

Up until now, studies were conducted to compare the quality characteristics and antioxidant properties of green teas according to the different brewing methods (Lee et al. 2013), according to the various degree of fermentation (Yanagimoto et al. 2003) and the different grade of Japanese green tea (Shimoda et al. 1995). To our knowledge, only limited scientific information on quality differences caused by different tea harvesting time is available although consumers pay different price based on it. Thus, this study was designed to find out how tea harvesting time affects the quality characteristics of green teas including polyphenolic composition, volatile compounds profile, and sensorial quality. Volatile and non-volatile compound analyses of 4 green tea leaf samples collected *different harvesting time were fully conducted and also the correlation of instrumental volatile and non-volatile compound analyses results and human sensory perception was conducted.*

Materials and methods

Green tea samples included in this study

Green tea samples were purchased through direct contact with supplier (Bosung Jeda, Bosung, Korea). A city of Bosung in Korea is well-known for green tea production in Korea, along with Jeju Island. All green tea samples included in this study were from the same suppliers, in which own their own field that have grown *Camellia sinensis* for more than 10 years, hence have strict quality standard to keep the product consistency. This geographical specificity provides the unique, but representative tea leaves that the only difference among samples were the harvesting time of tea leaf. All were harvested between April and May in 2014, and kept at 4 °C till processing at a designated local processing plant. Four green tea samples included Woojeon, Gokwoo, Sejak, and Choongjak, and their harvesting information can be found in Table 2.

Sample preparation for instrumental and sensory analysis

For the instrumental analyses, tea was brewed by pouring 190 mL of hot water (90 °C) to a beaker containing 10 g of

each green tea leaf as described by Kim et al. (2011) with minor modification. The slurries were stirred for 5 min prior to filtering through cheese cloth followed by Whatman #4 filter paper. The filtered tea infusions were immediately frozen until instrumental analyses. For sensory analysis of green tea, leaves were infused according to ISO standard (ISO 3103:1980) with slight modification. A 6-g of green tea leaf was measured for brewing and the brewing was carried out in a small white porcelain teapot. About 350 ml of 70 °C water was added to teapot and brewed for 2 min. While it was being brewed, the pot was stirred 10 times. Approximately 30 ml of brewed tea was poured into 80 ml-opaque white paper cup (Jin-Kwang papers, Paju-si, Gyeonggi-do, South Korea) and served to consumers.

Quality characteristics of Green tea

The pH of the brewed tea was measured with a pH meter (SevenEasy™ S20, Mettler-Toledo, Columbus, OH, USA), after brewing 6-g of green tea leaves in 350 ml of 70 °C water for 2 min. Brix and metal contents were measured by Rudolph J157 automatic refractometer at 25 °C (Hackettstown, NJ, USA) and by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy) as previously described by Fernández-Cáceres et al. (2001), respectively with the tea infusions brewed for the instrumental analyses. The color (L^* , a^* , b^* values) of 10-g powdered tea leaves was measured in triplicated by colorimeter (NE 4000, Nippon Denshoku, Osaka, Japan).

Total Chlorophyll content was measured by spectrophotometer following previous work (Kuroda and Hara 1999). Briefly, tea leaves 0.2 g were extracted in a colored glass with 50 ml of 85% acetone for 30 min. After the extraction, the tea was filtered through Whatman No. 2 filter paper into a colored volumetric flask and made to 50 ml using 85% acetone. Then, the absorbance was measured using Spectrophotometer at 663 and 645 nm, and the total chlorophyll content was calculated $20.9 \times A_{645} + 8.02 \times A_{663}$, and were reported as mg of chlorophyll per 1 L.

Non-volatile component analysis by HPLC–UV and antioxidant capacity determination by DPPH analysis

Individual polyphenolics (gallic acid and tea catechins) and methylxanthines (caffeine and theobromine) were analyzed by HPLC–UV analysis. Each tea infusion (brewed for the instrumental analysis) was properly diluted by reverse-osmosis water and filtered through a 0.45 µm PTFE disc filter (Whatman, Clifton, NJ, USA) prior to injection. Polyphenolic separation was conducted on an Agilent 1200 system

equipped with UV detector with a Luna 3 μ C18 (2) 100A 100 $\times$ 4.6 mm (3 micron) column run at 1.0 mL/min. The temperature of the column department was kept at 25 °C. A gradient mobile phase that consisted of A (98:2:1:0.1 = water:acetonitrile:methanol:water, v:v:v:v), B (100% acetonitrile), and C (100% methanol) was used for polyphenolic and methylxanthine separation. Gradient system is illustrated in Table 1. Phenolic compounds and methylxanthines were detected and quantified at 279 nm against external standards of gallic acid, theobromine, theophylline, (–)-epicatechin (EC), (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-gallocatechin gallate (GCG), and caffeine, all procured from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO) and Chromadex (Colorado, CA). The coefficients of determination (R^2) were determined and they were all higher than 0.999 for calibration curves of all analytical standards used in this study. The DPPH radical scavenging activities was applied to measure the antioxidant activities of green tea samples as described by Atoui et al. (2005) and Siddhuraju and Becker (2003) after minor modification.

Evaluation of volatile compounds by GC/MS

Volatile analysis was performed as described by Kim et al. (2011) on the brewed tea used for the instrumental analysis. The tea samples were prepared for analysis by adding 4.95 ml of diluted teas and 50 μ L of internal standard (phenol-D6, 1000 ppm) into a 20 ml amber headspace vial. For sample preparation, 2 cm 3-phase SPME fiber with Divinylbenene, Carboxen, Polydimethylsiloxane were utilized in a Gerstel Multi-Purpose Sampler (MPS-2) (Baltimore, MD). The fiber was selected because the preliminary studies showed the superior consistency and recovery using 3-phase SPME fiber. The fiber was conditioned at 270 °C for 30 min before use. A 10-min incubation followed by a 40 min exposure was used to capture the volatiles on the fiber for injection into the GC. The samples were stirred using a 3 $\times$ 12 mm stir bar in the 20 ml vial, then desorbed

for 5 min, at the GC injector port. An Agilent 7890A gas chromatograph (Palo Alto, CA) was used for the analysis with splitless mode. The flow rate of helium, as a carrier gas, was set at 1.25 ml/min through a 60 m $\times$ 0.25 mm $\times$ 0.25 μ m RTX-5 ms column. The initial oven temperature was 50 °C immediately followed by a 4 °C/min temperature ramp to 170 °C which was followed by a 100 °C/min ramp to 250 °C and held for 5 min. The transfer line was held at 250 °C to LECO TruTOF MS (St. Joseph, MN). Data was collected for 20–250 m/z at an acquisition rate of 10 spectra per second. Compounds were identified by spectral matches based on the NIST14 database, retention indices, and by comparison to authentic standards. Peak areas were normalized to the internal standard response for each analysis for statistical analyses.

Consumer acceptance test

All four green tea samples were evaluated for consumer acceptability. A total of sixty-six green tea consumers were recruited through Dongguk University-Taste tester database consisting of about 150 active participants from Department of Food Science and Biotechnology at Dongguk University. Participants were initially asked to fill out a brief questionnaire containing demographic questions. Once demographic questions were filled out, green tea samples brewed as described above, were presented to participants. The samples were blind-coded with 3-digit random numbers. Samples were served in monadic presentation, therefore the participants were not able to taste of green tea samples back and forth. The order of sample presentation was randomized according to William's Latin-square design, and samples were served to participants at 45 $\pm$ 2 °C, as described in Lee and Chambers (2011). Participants were asked to evaluate the appearance, and flavors. Participant first evaluated appearance, color, taste and overall liking. They were also asked to evaluate other attributes including bitterness and astringency. Open-ended questions were also asked for their liking and disliking of samples. The attributes were evaluated using a 9-point hedonic scale and anchored in Korean, with one being dislike extremely, and nine being like extremely.

Statistical analysis

Data obtained from four different green tea evaluated in triplicate were analyzed by ANOVA using JMP software, version 5 (SAS, Cary, NC) and mean separation determined by the LSD test ($p < 0.05$). Partial Least Square (PLS) analysis was conducted in order to predict the volatile and non-volatile compounds responsible for consumer overall liking of green tea samples, using XLSTAT (ver. 2014. Addinsoft, Paris, France).

Table 1 HPLC gradient system for methylxanthin and polyphenolic separation

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0	100	0	0
8	90	0	10
14	70	15	15
20	70	20	10
25	60	35	5
32	45	45	10
35	100	0	0
38	100	0	0

Table 2 Quality characteristics of 4 green tea samples included in this study

Sample	Harvesting time (Date)	PH	°Brix	L*	A*	B*	Total chlorophyll content (mg/L)
Woojeon	4.1.14–4.20.14	6.56 ± 0.02 b	2.34 a	41.3 ± 7.1 a	−2.3 ± 0.9 a	18.2 ± 1.3	7.95 ± 0.01 b
Gokwoo	4.20.14–4.30.14	6.59 ± 0.02 b	2.26 b	36.4 ± 4.7 a	−1.7 ± 0.7 a	17.0 ± 1.2	7.58 ± 0.01 c
Sejak	5.1.14–5.15.14	6.56 ± 0.02 b	2.26 b	40.1 ± 6.8 a	−2.1 ± 0.6 a	16.5 ± 0.7	6.83 ± 0.02 d
Choongjak	5.16.14–5.30.14	6.66 ± 0.03 a	2.20 c	36.3 ± 2.2 a	−2.1 ± 0.4	15.8 ± 1.3	8.56 ± 0.01 a
<i>p</i> value		0.001	<0.05	0.610	0.714	0.135	<0.0001

Different letters within column indicate significant differences at $p < 0.05$ L*, a* and b* values are the name of what we measure the color

Results and discussion

Quality characteristics of green tea

Harvesting time of tea leaf is one of the most important factors influencing the physical and chemical qualities of tea along with geographical difference and processing method. However, very limited information on quality differences caused by different tea harvesting time was available. Thus, differences in quality parameters induced by several harvesting time such as LAB color, pH, soluble solid (°Brix), and metal (mineral) content were evaluated (Table 1). Overall, no noticeable difference was observed in the color (LAB) of each infusion and it was confirmed by visual inspection and statistical analysis. The pH of every tea infusion was falling within normal range of typical brewed green tea (pH 6.0 ~ 7.0) but the pH of earlier harvested tea infusions (Woojeon, Gokwoo, and Sejak) were lower than that of Choongjak, which was harvested the last ($p < 0.05$). Green tea catechins are more stable at lower pH (Chen et al. 2001), so it kept catechins better from degradation during hot water brewing and air exposure, which may resulted in higher quality tea infusion in terms of health benefits. Brix also showed the similar trend with pH difference as earlier harvested tea contained more tea solid, which was known to provide astringency/bitterness to the body of tea infusion. Typical composition of soluble solid (°brix) in tea included tasting compounds such as polyphenolics, catechin, protein, and carbohydrate (Harbowy and Balentine 1997). Thus, this higher soluble solid could be partly because higher polyphenolic content in early harvested tea as observed in non-volatile compound analysis. Total chlorophyll content was the highest in Choongjak because it had enough time for the development of chlorophyll before harvest in mid-May. Chlorophyll being water-insoluble compound, was not extracted by traditional hot water brewing method. Thus, chlorophyll content in this study was the only quality characteristics that evaluate tea leaves, rather than tea infusion. Mineral content of tea has been used as one of important chemical parameters between tea varieties because it was directly related to the taste of tea (Alcazar

Table 3 Metal contents in 4 green teas measured by ICP-AES

Green tea	Potassium	Magnesium	Calcium	Sodium
Woojeon	952 ^a	74	<17	24
Gokwoo	932	68	<17	<17
Sejak	932	69	<17	<17
Choongjak	868	60	<17	<17

^a Units are expresses as mg/L

et al. 2007; Chaturvedula and Prakash Chaturvedula and Prakash 2001). Potassium was identified as the most prevalent metal ion in all 4 green tea sample and it was in agreement with previous report (Yemane et al. 2008). As for Magnesium, Woojeon had higher magnesium content than that of other tea (Table 3).

Non-volatile components

Flavan 3-ols (catechins) are known to contribute the antioxidant properties, and *camellia sinensis* teas (green tea) are well-known for rich source of those compounds (Chen et al. 1998; Fernandez et al. 2000). In addition, methylxanthins such as theobromine, theophylline, and caffeine are naturally present in *camellia sinensis* tea. Among all the compounds under methylxanthin group, caffeine was believed to be the most important methylxanthin compound (Marcia et al. 2002). The only non-catechin polyphenolic compound found in the green teas was gallic acid and its concentration was higher in earlier harvested tea (Table 4). As a naturally occurring phenolic compound in tea, gallic acid contributes to antioxidant capacity of tea and results in higher quality of tea that provides potential health benefits to tea drinkers (Kin et al. 2011).

Total 5 tea catechins were detected by HPLC–UV analysis in the green tea infusions and they were identified as EGC (epigallocatechin), EGCG (epigallocatechin gallate), EC (epicatechin), GCG (gallocatechin gallate), and ECG (epicatechin gallate) by comparing the retention time and characteristic spectra of pure analytical standards. Overall, the most prevalent catechin and the strongest

Table 4 Individual polyphenolics, methylxanthines, and total antioxidant activities of 4 green teas used in this study

No.	Green tea	Gallic acid	Theobromine	EGC ^a	Caffeine	EGCG	EC	GCG	ECG	Total catechin ^a	Total antioxidant capacity ^b
1	Woojeon	1050.0 ± 2.8 ^a	205.7 ± 27.1 ^a	10199 ± 652.6 ^a	4159 ± 17.1 ^a	9589.2 ± 286.4 ^a	1018 ± 56.1 ^a	35.0 ± 4.1 ^b	2322 ± 59.2 ^a	23,164 ± 48.9 ^a	61.05 ± 0.27 ^a
2	Gokwoo	800.4 ± 8.6 ^b	151.5 ± 24.4 ^b	7210 ± 772.2 ^b	3821 ± 28.9 ^b	8999.4 ± 858.5 ^a	711.7 ± 51.2 ^b	59.5 ± 8.6 ^b	2139 ± 31.2 ^b	19119 ± 60.6 ^b	58.92 ± 0.57 ^{ab}
3	Sejak	675.9 ± 28.4 ^c	120.8 ± 2.5 ^b	4527 ± 93.2 ^c	3687 ± 46.4 ^c	8677.1 ± 723.8 ^{ab}	604.3 ± 44.6 ^c	52.9 ± 5.4 ^b	2012 ± 27.2 ^c	15873 ± 53.2 ^c	61.04 ± 0.42 ^a
4	Choongjak	419.4 ± 6.0 ^d	75.71 ± 3.3 ^c	2342 ± 50.4 ^d	3409 ± 47.2 ^d	7111.9 ± 1406.9 ^b	321.2 ± 30.0 ^d	266.2 ± 43.9 ^a	1920 ± 75.2 ^c	11961 ± 31.5 ^d	55.12 ± 0.42 ^b

Units are expressed as mg/L

EGC epigallocatechin, EGCG epigallocatechin gallate, EC epicatechin, GCG gallocatechin gallate, ECG epicatechin gallate

^a Total catechin includes EGC, EGCG, EC, GCG, and ECG^b Total antioxidant capacity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) method

Different letters within column indicate significant difference

antioxidant compound in tea, EGCG, was higher in earlier harvested tea compared to Choongjak that was harvested as the best, and this was in agreement with previous finding (Rice-Evans 1999). In general, the earlier tea was harvested, the more individual catechins were detected in the tea infusion with exception of GCG. Total catechin content including all five detected catechins also showed the same trend in that the concentration in Woojeon (earlier harvest tea) was higher by 14, 27, and 43% than that of Gokwoo, Sejak, and Choongjak, respectively.

Tea catechins can be classified into two categories: catechins without galloyl moieties (EGC and EC) and catechins with galloyl moieties (EGCG, GCG, and ECG). The catechin concentration without galloyl moieties such as EGCG, GCG and ECG showed inverse relation to the maturity of tea leaf. The concentrations of EGC were 44.0, 37.7, 28.5, and 19.6% and those of EC was 4.40, 3.72, 3.81, and 2.69% in Woojeon, Gokwoo, Sejak, and Choongjak, respectively. On the other hand, the catechin concentration with galloyl moieties was elevated while tea leaf was still growing in the soil before harvesting. The concentrations of EGCG were 41.4, 47.1, 54.7, and 59.5%, those of GCG was 0.15, 0.31, 0.33, and 2.23%, and those of ECG were 10.0, 11.2, 12.7, and 16.1 in Woojeon, Gokwoo, Sejak, and Choongjak, respectively. Consistently, gallic acid concentration increased with tea maturity, and it could be because of the fact that gallic acid concentration is naturally higher in early stage of development, and/or free gallic acids were released during tea maturation from catechins with galloyl moieties. Concentrations of all tea catechins with galloyl moieties such as EGCG, GCG, and ECG decreased, while % of tea catechins without galloyl moieties such as EGC and EC increased. This might be positive property for earlier harvested teas because antioxidant capacity of catechins with galloyl moieties were generally higher than the ones without galloyl moieties (Rice-Evans 1999; Hilal and Engelhardt 2007). Two detected methylxanthines, theobromine and caffeine, were also higher in earlier harvested tea. Total antioxidant capacity that was affected by polyphenolic activity also showed similar trend in that the tea harvested earlier and showed higher concentration of polyphenolics in each tea infusion.

The Woojeon tea is considered as the highest quality green tea variety in Korea, and literally means “teas harvested before Gokwoo”. Gokwoo is the time for the rain to fall between early April and early May for planting of spring cereal grains”. Since the Woojeon tea leaf was young and soft and its physical and chemical properties were strikingly similar with those of white tea which was harvested when the leaf was still young. It has been reported that the polyphenolic concentration, catechin content, and caffeine were higher in white tea leaf

Table 5 Name, retention index, relative concentration, and odor descriptor of volatile compounds detected in four green teas by GC/MS analysis

No.	Group	Compound name	Retention index	Woojeon	Gokwoo	Sejak	Choongjak	Odor descriptor
1	Sulfur	Dimethyl sulfide	469.1	47 ^a	552	75	100	Cabbage, gasoline, organic, sulfur, wet earth
2	Aldehydes	2-Methylpropanal	517.0	41	19	53	100	Burnt, caramel, cocoa, green, malt
3	Aldehydes	3-Methylbutanal	550.0	71	37	127	100	Almond, cocoa, fresh green, malt, pungent
4	Aldehydes	2-Methylbutanal	649.8	ND	19	55	100	Almond, cocoa, fermented, hazelnut, malt
5	Alcohols	1-Penten-3-ol	660.4	42	9	72	100	Butter, fish, green, oxidized, wet earth
6	Ketones	1-Penten-3-one	326.0	ND	ND	ND	100	Fish, green, metal, mustard, pungent
7	Aldehydes	Pentanal	685.9	316	83	239	100	Almond, bitter, malt, oil, pungent
8	Aldehydes	(E)-2-Pentenal	699.2	ND	ND	ND	100	Paint
9	Aldehydes	Hexanal	753.5	1057	1017	1198	100	Apple, fat, fresh, green, oil
10	Aldehydes	2-Hexenal	802.0	ND	ND	9	100	Fat, rancid
11	Ketones	2-Heptanone	892.3	85	25	126	100	Blue cheese, fruit, green, nut, spice
12	Aldehydes	(Z)-4-Heptenal	902.1	ND	ND	11	100	Biscuit, cream, fat, fish, rotten
13	Aldehydes	Heptanal	903.7	142	25	98	100	Citrus, fat, green, nut, rancid
14	Aldehydes	Benzaldehyde	968.2	236	26	42	100	Bitter almond, burnt sugar, cherry, green almond, roasted pepper
15	Aldehydes	6-Methyl-5-hepten-2-one	990.5	47	4	62	100	Citrus, mushroom, pepper, rubber, strawberry
16	Hydrocarbons	Myrcene	993.5	122	ND	35	100	Balsamic, fruit, geranium, herb, must
17	Ketones	2-Octanone	1002	ND	ND	ND	100	Fat, fragrant, gasoline, mold, soap
18	Aldehydes	(E,E)-2,4-Heptanedienal	1006	ND	107	494	100	Fat, nut, plastic
19	Aldehydes	Octanal	1016	8	4	ND	100	Citrus, fat, green, oil, pungent
20	Aldehydes	Benzeneacetaldehyde	1052	1	ND	ND	100	Berry, geranium, honey, nut, pungent
21	Aldehydes	(E)-2-Octenal	1062	ND	10	ND	100	Dandelion, fat, fruit, grass, green, soap, spice
22	Ketones	(E,E)-3,5-Octadien-2-one	1068	ND	17	62	100	Fat, fruit, mushroom
23	Alcohol	Linalool	1105	ND	ND	129	100	Coriander, floral, lavender, lemon, rose
24	Aldehydes	Nonanal	1108	200	84	91	100	Fat, floral, green, lemon, paint
25	Alcohols	Hotrienol	1109	92	13	106	100	Fresh, hyacinth, lemon, sweet
26	Aldehydes	Safranal	1213	39	32	ND	100	Herb, sweet

^a Relative concentration was calculated by comparing with Choongjak green tea

compared to green tea leaf when about 30 samples of each green and white tea were evaluated (Komes et al. 2009). According to Komes et al. (2009), caffeine content was also higher in white tea compared to other *Camellia sinensis* based tea including green tea, oolong tea, and black tea. Previous work also reported the higher levels of theanine, theobromine, caffeine, and total catechin contents in earlier harvested green tea, such as Woojeon and gokwoo (Lee et al. 2014). These observations were in agreement with the result obtained in the present study and it indicated that caffeine and polyphenolic compounds in the tea leaf developed in the early stage of growth and then degraded while the tea leaf was growing in the field.

Volatile compound analysis

While volatile compounds take only about 0.01% of total dry tea weight, but their contribution to the overall quality of tea was significant (Pripdeecech and Wongpornchai 2013). Total 26 volatile compounds were identified by GC/MS using SPME extraction. Their retention indices, relative concentrations (%), and odor descriptors are displayed in Table 5. The compounds have “green” odor such as 2-methylpropanol, 3-methylpropanol, 1-penten-3-ol, 1-penten-3-one, hexanal, 2-hepanone, heptanal, benzaldehyde, Octanal, (E)-2-octenal, and nonanal were detected and this green aroma was generally higher in Choongjak green tea

with only exception of hexanal. Hexanal was considered as an universal aroma compound in plant kingdom. This indicated that both “green” volatile (11 green volatile compounds) and non-volatile (chlorophyll) constituents in green tea are continuously developed throughout green tea’s growing period and reaches a peak late May.

Of 26 identified volatile compounds in green tea, sixteen of them were aldehyde group, five compounds were ketone group, and three volatiles were alcohol group, and one of each sulfur compound and hydrocarbon was also detected. Linalool that has floral and lemon aroma was only detected in Sejak and Choongjak, which indicated that this compound was formed at later stage of growth, while nonanal, another floral aromatic compound, decreased after the early stage. Many green tea volatile compounds were only or predominantly found in Choongjak since they were synthesized at the late stage of tea leaf development. These compounds were 2-methylpropanal, 2-methylbutanal, 1-penten-3-one, (E)-2-pentenal, 2-Hexenal, (Z)-4-heptanone, 2-octanone, octanal, benzeneacetaldehyde, (E)-2-octenal, (E,E)-3, 5-octadien-2-one, and safranal and they were either aldehydes or ketones. Otherwise, compounds like pentanal and hexanal tend to be reduced while tea leaf was growing, so their characteristic oil-like flavor might have been reduced. Even though overall flavor compound concentration was the highest in Choongjak, it is hard to conclude that Choongjak was best flavor tea because threshold of each compound is different and some volatile compounds gave negative impact on overall flavor profile.

Consumer acceptability

In order to understand how these differences in physiochemical quality characteristics as well as volatile and non-volatile characteristics of four green tea samples influences the consumer likings of green tea samples, consumer acceptance test was performed. No significant differences were observed in the appearance, color and flavor liking attributes among 4 green tea samples ($p > 0.05$). Overall liking scores of 4 green tea samples ranged from 4.3 to 5.9 (Table 6) (Chung and Han 2013). Interestingly Woojeon,

which was believed to be the highest quality green tea product in Korea, received the lowest overall liking score ($p < 0.05$). Woojeon received lowest bitter taste and astringency liking scores of 4.2 and 4.1, respectively. This suggested that the bitterness and astringent mouthfeel may have influenced the mean drop on overall liking of Woojeon products. The consumers’ responses collected from the open-ended questions on Woojeon included “objectionably strong bitter taste (27.6%)”, and “too astringent (13.4%).” The comments collected from open-ended question and the liking scores collected from overall liking, bitterness and astringency likings for Woojeon were in agreement with each other. Sejak and Choongjak received the highest overall liking scores, which suggested that these two products were well accepted by consumers than Woojeon. The consumer liked Choongjak because of lower bitterness (22.3%) and low intensity of green tea flavor (18.7%). While no differences were observed in instrumental color measurement, consumers also noted the slight difference in color of Choongjak product which was lighter than other products. Finding from current work was also in agreement with previous work in that consumers liked green tea with low flavor bitterness and astringency (Lee and Chambers 2011).

Correlating volatile, and non-volatile compounds to consumer liking of green tea

Partial Least Square (PLS) analysis was conducted in order to predict the volatile and non-volatile compounds responsible for consumer overall liking of green tea samples (Fig. 1). The loading plot (Fig. 1) visualized how 4 green samples were located in the volatile and non-volatile attribute map, and provided the visual investigation of the volatile and non-volatile attributes that influenced consumer preference. According to the PLS loading plot, the volatile and non-volatile attributes positively influences consumer liking included linalool, 2/3-methyl butanal, 2-heptanone, (E,E)-3,5-Octadien-2-one. Linalool was known to have characteristic floral/lemon aromatic, and it showed high correlation to overall liking to green tea

Table 6 Consumer acceptance scores of 4 green tea samples (N = 66)

	Appearance liking	Color liking	Overall liking	Flavor liking	Bitter taste liking	Astringency liking
Woojeon	5.7 ± 0.2 a	5.6 ± 0.2 a	4.3 ± 0.3 b	5.3 ± 0.2 a	4.2 ± 0.3 b	4.1 ± 0.1 b
Gokwoo	6.1 ± 0.3 a	6.1 ± 0.3 a	5.1 ± 0.4 ab	5.8 ± 0.3 a	4.9 ± 0.4 ab	4.8 ± 0.1 ab
Sejak	6.2 ± 0.2 a	6.2 ± 0.3 a	5.6 ± 0.4 a	5.9 ± 0.3 a	5.3 ± 0.4 a	5.1 ± 0.1 a
Choongjak	5.5 ± 0.3 a	5.7 ± 0.2 a	5.9 ± 0.4 a	5.9 ± 0.3 a	5.6 ± 0.4 a	5.4 ± 0.1 a
<i>p</i> value	0.049	0.091	<0.0001	0.108	0.002	0.003

Means in a same column that does not share the same alphabetical letter represent the significant differences ($p < 0.05$)

Fig. 1 PLS analyses on the instrumental volatile and non-volatile analyses and consumer overall liking

product. 3-methyl butanal and 2-methyl butanal were known to have characteristic malty/nutty aromatics, 2-heptanone is known for fruity, green, spice aromatic, and (E,E)-2,4-Heptanedienal has fruity and mushroom aroma characteristics. All five compounds positively influenced the consumer liking of green tea products having characteristics fruity and malty aroma, and were found in high concentration on Sejak and Choongjak (Table 4). Choongjak correlated well with a variety of volatile compounds than other products, suggesting the versatile flavor characteristics of Choongjak than others, which were developed during the last stage of development. Volatile compounds (nonanal, benzaldehyde and heptanal) correlate well with Woojeon and these compounds were known to have characteristic green, rancid/nutty aroma. As for non-volatile attributes, the earlier harvest green teas (Woojeon and Gokwoo) correlate well with non-volatile constituents including gallic acid, theobromine, and different types of catechins (EGC, EGCG, EC, GCG, ECG, and total catechin), and subsequently °Brix content. However these non-volatile constituents were identified as driver of consumer disliking of green tea products. Previous work also reported that the catechins influenced the overall flavor and

provided bitter and astringency characteristics of green tea (Lee and Chambers 2011; Saklar et al. 2015). The finding from current work also support the previous findings.

Conclusion

The influence of tea leaf harvesting time on the chemical and physical parameters of green teas were investigated in this study by analyzing quality characteristics, polyphenol composition, volatile components, and further correlated these characteristics to human hedonic perceptions. Overall, green tea harvested earlier (Woojeon) had higher antioxidant capacity due to high catechin content. However, high catechin content in green tea influenced negatively the consumer acceptance of green tea due to high intensities of bitterness and astringency. Volatile compounds drive consumer liking of green tea products were also identified that included linalool, 2/3-methyl butanal, 2-heptanone, (E,E)-3,5-Octadien-2-one. Current work demonstrated the quality differences as well as consumer acceptability of green tea collected in different harvesting

time. Finding from current study can practically aid green tea industry for selection of green tea leaf for further flavor development which required a fine balance between antioxidant benefits and consumer likings.

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